

### **In the Claims**

1-17. (Cancelled)

18. (New) A method for quantitative measurement of gene expression of a target gene in a fixed paraffin embedded tissue sample comprising:

(a) deparaffinizing the tissue sample to obtain a deparaffinized sample;

(b) isolating mRNA from the deparaffinized sample by first heating the tissue sample in a chaotropic solution comprising an effective concentration of a chaotropic agent to a temperature in the range of about 50 to about 100°C for a time period of about 5 to about 120 minutes and recovering said mRNA from said chaotropic solution to yield isolated mRNA; and

(c) subjecting the isolated mRNA to amplification using a pair of oligonucleotide primers capable of amplifying a region of the target gene mRNA, to obtain an amplified sample;

(d) determining the quantity of target gene mRNA relative to the quantity of an internal control gene's mRNA from the isolated mRNA.

19. (New) The method of claim 18 further comprising rehydrating the sample before heating.

20. (New) The method of claim 19 further comprising homogenizing said sample before heating.

21. (New) The method of claim 18 wherein the isolated mRNA is recovered with a water insoluble organic solvent.

22. (New) The method of claim 21 wherein said water insoluble organic solvent comprises chloroform.

23. (New) The method of claim 18 wherein the isolated mRNA is recovered from said chaotropic solution by alcohol precipitation.

24. (New) The method of claim 18 wherein said time period of heating is from about 10 to about 60 minutes.

25. (New) The method of claim 24 wherein said time period of heating is from about 30 to about 60 minutes.

26. (New) The method of claim 18 wherein said temperature is in the range of about 85 to about 100° C.

27. (New) The method of claim 26 wherein said time period is from about 30 to about 60 minutes.

28. (New) The method of claim 18 wherein said chaotropic agent is a guanidinium compound.

29. (New) The method of claim 28 wherein said guanidinium compound is guanidinium hydrochloride.

30. (New) The method of claim 18 wherein said guanidinium compound is guanidinium isothiocyanate.

31. (New) The method of claim 30 wherein said guanidinium isothiocyanate is present in a concentration of about 2 to about 5 M.

32. (New) The method of claim 31 wherein said guanidinium isothiocyanate is present in a concentration of about 4M.
33. (New) The method of claim 28 wherein said chaotropic solution has a pH of about 3 to about 6.
34. (New) The method of claim 33 wherein said chaotropic solution has a pH of about 4.
35. (New) The method of claim 18 wherein said chaotropic solution further comprises a reducing agent.
36. (New) The method of claim 35 wherein said reducing agent is  $\beta$ -mercaptoethanol.
37. (New) The method of claim 35 wherein said reducing agent is dithiothreitol.
38. (New) The method of claim 18, wherein the tissue sample is formalin-fixed and paraffin embedded (FFPE).
39. (New) The method of claim 18 wherein determining the relative gene expression level is determined using RT-PCR.
40. (New) The method of claim 18 wherein the relative quantity of target gene mRNA in said tissue sample is the same whether said tissue sample is formalin-fixed and paraffin embedded or frozen.

41. (New) The method of claim 18 wherein, the internal control gene is  $\beta$ -actin.

42. (New) A method for determining the level of a target gene expression in a fixed paraffin embedded tissue sample comprising:

(a.) deparaffinizing the tissue sample to obtain a deparaffinized sample;

(b.) isolating mRNA from the deparaffinized sample by first heating the deparaffinized tissue sample in a solution comprising an effective concentration of a chaotropic agent to a temperature in the range of about 50 to about 100° C and recovering said mRNA from said solution; and

(c.) determining the quantity of the target gene mRNA relative to the quantity of an internal control gene's mRNA.

43. (New) The method of claim 42, wherein the heating takes place from about 5 to about 120 minutes.

44. (New) The method of claim 42 wherein determining the relative gene expression level comprises RT-PCR.

45. (New) A method for quantitative measurement of gene expression of a target gene in a fixed paraffin embedded tissue sample comprising:

(a) deparaffinizing the tissue sample to obtain a deparaffinized sample comprising DNA and RNA;

(b) isolating mRNA from the deparaffinized sample by first heating the tissue sample in a solution comprising an effective concentration of a chaotropic agent to a temperature in the range

of about 50 to about 100°C for a time period of about 5 to about 120 minutes and recovering said mRNA from said chaotropic solution to yield isolated mRNA; and

(c) subjecting the isolated mRNA to amplification using a pair of oligonucleotide primers capable of amplifying a region of the target gene mRNA, to obtain an amplified sample;

(d) determining the quantity of target gene mRNA relative to the quantity of an internal control gene mRNA from the isolated mRNA.

46. (New) The method of claim 45 wherein recovering the isolated mRNA comprises a recovery technique selected from the group consisting of extraction, electrophoresis, chromatography, precipitation, and combinations thereof.

47. (New) The method of claim 45 wherein determining the relative gene expression level comprises RT-PCR.

48. (New) The method of claim 18 wherein the mRNA is isolated free of DNA present in the sample.

49. (New) The method of claim 45 wherein the mRNA is isolated free of DNA present in the sample.